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Blue emitting gold nanoclusters templated by poly-cytosine DNA at low pH and poly-adenine DNA at neutral pH

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Blue fluorescent gold nanoclusters were prepared in the presence of poly-cytosine DNAs at low pH and poly-adenine at neutral pH using citrate as the reducing agent; various buffer conditions affecting the synthesis have been explored.

Few atom noble metal nanoclusters (NCs) have emerged as a useful class of materials for biosensing, imaging and catalysis.¹ These NCs possess discrete energy states similar to molecular fluorophores. Compared to semiconductor quantum dots, Au and Ag NCs have fewer toxicity concerns. Their synthesis usually requires strong polymeric or chelating ligands to avoid the growth of larger nanocrystals. AuNCs have been prepared in the presence of thiol compounds,² dendrimers,^{3–5} peptides,⁶ and proteins.⁷ Many AgNCs were obtained using cytosine-rich DNAs. Taking advantage of the binding interaction between silver and cytosine,⁸ a diverse range of emission colors have been achieved using different DNA sequences.⁹ DNA templated synthesis has a number of advantages. First, in addition to serving as a template, DNA possesses functional roles for molecular recognition (e.g. aptamers).¹⁰ With appropriately designed DNA sequences, it is possible to directly couple analyte binding with fluorescence signaling.¹¹ Second, since DNA is much smaller compared to proteins, the associated NCs are not deeply shielded, allowing more effective energy transfer to other fluorophores and quenchers. Third, chemical synthesis of DNA allows for a large degree of versatility in DNA sequence and modification, facilitating fundamental studies and practical applications. Finally, unlike many synthetic polymers, DNA is non-toxic with good biocompatibility.

Compared to AgNCs, AuNCs might be more stable since gold is less easily oxidized.¹² However, DNA-templated AuNCs have not been reported. Based on current knowledge, DNA needs to bind strongly to gold precursors and AuNCs (Figure 1A). The most commonly used gold source is HAuCl₄. Since DNA, AuCl₄ and gold nanoparticles are negatively charged, we reason that a favorable interaction might be achieved at low pH.¹³ In addition, the ratio between gold and DNA may also be important.

Since there is almost no information on the interaction between DNA and AuNCs, we first tested several DNA homopolymers; A₃₀, T₃₀ and C₃₀ were chosen for this purpose. The reaction was carried out using 100 μM HAuCl₄ and 50 μM DNA at pH 3–7 using citrate as buffer and reducing agent. After overnight incubation, C₃₀ produced blue fluorescent AuNCs under 245 nm UV light excitation (Figure 1B). The fluorescence was more intense with lower pH and the fluorescence excitation spectrum shows two peaks at 275 nm and 360 nm (Figure 1D). Since exciting either peak generated the identical emission spectral shape, both wavelengths should excite the same electronic transition of the AuNC. Since the absorption maximum of poly-C DNA is at 275 nm, it is likely that the 275 nm excitation peak is due to energy transfer from cytosine to AuNC,¹⁴ which also suggests that the AuNCs were associated with the DNA. Therefore, the direct excitation wavelength should be 360 nm, yielding a Stokes shift of ~80 nm for the 440 nm emission peak, which is consistent with the literature reported value for blue emitting AuNCs.³ Its UV-vis absorption spectrum also shows a strong peak at 360 nm due to AuNC absorption (see Figure S1 of ESI). There is also a very weak surface plasmon band at ~540 nm. Transmission electron microscopy data showed that the maximal Au size is ~2 nm (Figure S2), consistent with the weak surface plasmon peak. Blue emitting AuNCs are too small to be resolved.

Using 275 nm excitation, the emission spectra from different DNA sequences at pH 3 were collected (Figure 1E). A₃₀ produced very weak fluorescence whose intensity was only ~10% of that with C₃₀, while T₃₀ produced almost no fluorescence. The samples were also observed under ambient light and we found that some of the T₃₀ samples were red (Figure 1C, e.g. from pH 4–6), indicating the formation of relatively large AuNPs exhibiting the surface plasmon effect. In the absence of DNA, no fluorescent AuNCs were produced while purple Au nanoparticles were found to stick to the tubes (see arrow heads of Figure 1C). Therefore, DNA sequence and pH are important for producing fluorescent AuNCs. Poly-A DNA is known to interact with the AuNP surface strongly.^{15,16} Although A₃₀ failed to produce fluorescent AuNCs in this case, a careful optimization of the ratio between Au and DNA yielded highly fluorescence AuNCs (*vide infra*).

To quantitatively test the effect of pH, we diluted the AuNCs in Figure 1B with water and the highest fluorescence was observed at pH 3 (Figure 1F). Reducing pH further to 2 showed slightly reduced emission. Fluorescence dropping and emission peak broadening were observed at higher pH. C-rich DNAs have been often used to prepare fluorescent AgNCs at neutral pH since Ag⁺ can tightly bind to the N3 nitrogen of cytosine.⁸ Crystallographic data also showed a similar binding between AuCl₄⁻ and 1-methylcytosine.¹⁷ Since the *pK_a* of cytosine is 4.2, the N3 position is protonated at pH 3 and is unlikely to contribute to gold binding. Binding of copper by the keto oxygen of cytosine at low pH was observed,¹⁸ which may also explain the binding of gold in our work (Figure S3). The above fluorescence measurements were performed at the pH where AuNCs were synthesized. We next diluted AuNCs from the same stock sample into buffers at various pH (Figure 1G). The fluorescence intensity barely changed from pH 3 to 6, and a drop was observed at higher pH, indicating that these C₃₀ templated AuNCs are highly stable at acidic conditions.

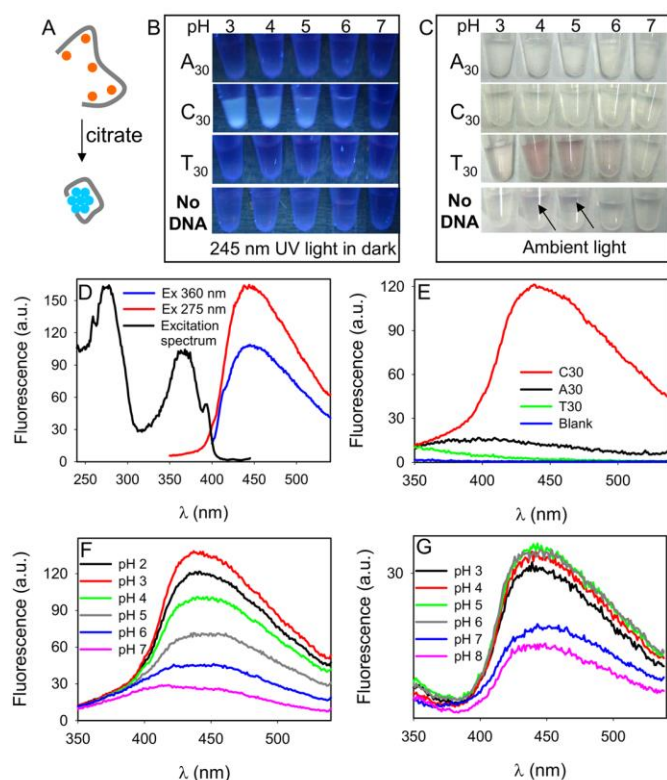


Figure 1. (A) A scheme of DNA templated AuNC synthesis. Fluorescence (B) and optical (C) graphs of DNA/HAuCl₄ mixture at various pH citrate buffer after overnight incubation. The arrow heads in (C) point to AuNPs sticking to the tube surface. (D) Fluorescence emission and excitation spectra of AuNCs templated by C₃₀. The feature at ~390 nm of the excitation spectrum is from water Raman scattering. Fluorescence spectra of AuNCs templated by various DNAs at pH 3 (E) or by C₃₀ at various pH (F). (G) AuNC fluorescence as a function of pH.

For most previous work, NaBH₄ was used as the reducing agent, which is unstable and needs to be freshly prepared. Alternatively, the native reducing property of proteins have also been harnessed.⁷ In this work, citrate was used and the kinetics of fluorescence generation was monitored. In the first 2 hrs, the signal reached ~70% of the final intensity and it took ~8 hrs to reach stable fluorescence (Figure S4). Next we studied the effect of citrate concentration (Figure 2A), where fluorescence decreased at lower citrate concentrations. Since citrate was used also as a buffer, to compensate the possible pH change, we tested another sample with 20 mM phosphate buffer (pH 3) and 1 mM citrate, which produced a stronger fluorescence intensity than the sample with just 1 mM citrate, but the intensity was still weaker than that with 50 mM citrate. Therefore, to achieve high fluorescence, a high citrate concentration is needed.

In the above work, the C₃₀:Au ratio was 1:2, which means that the cytosine: Au ratio was 15:1. To further optimize the synthesis, we fixed the HAuCl₄ concentration and varied the DNA added (Figure 2B). The higher the C₃₀ concentration, the stronger the fluorescence. This is different from the case of using C-rich DNA for AgNC synthesis, where a DNA:Ag⁺ ratio of 1:6 is often used (cytosine:Ag⁺ ≈ 2:1). Our experiment suggests that the binding between cytosine and gold at pH 3 might be weaker than that with Ag at neutral pH. We next prepared AuNCs and AgNCs using C₃₀ separately and the AuNCs were more resistant to photobleaching (Figure S5).

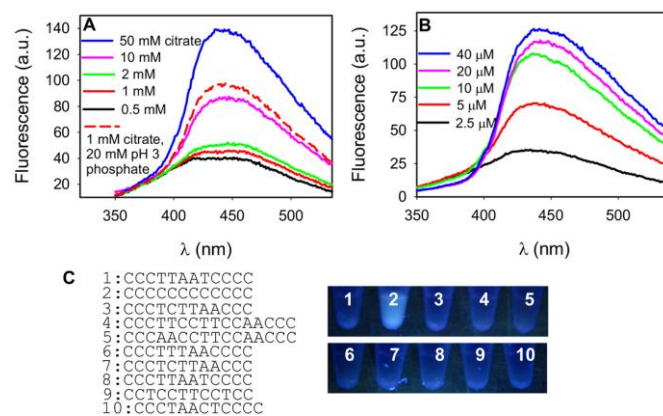


Figure 2. AuNC fluorescence spectra as a function of citrate concentration (A) and C₃₀ DNA concentration (B). (C) Photograph of AuNCs synthesized by various C-rich DNAs under UV excitation.

Since AgNCs with various emission colors were produced using different DNA sequences, to test whether this is true for AuNCs, we tested a number of C-rich DNAs that were reported to produce different AgNCs (Figure 2C).⁹ However, only DNA2 with its sequence being C₁₂ produced bright fluorescence (Figure S6), which was even stronger than that with C₃₀. This study shows that the optimal DNA should be short polycytosine.

Since A₃₀ also showed weak fluorescence in Figure 1E, next we aim to optimize its synthesis condition. Using 50 mM pH 7 citrate buffer, we fixed HAuCl₄ to be 150 μM and varied A₃₀ from 0.5 to 8 μM. The highest fluorescence was observed with 4 μM A₃₀ (Figure 3A), where the ratio between adenine and HAuCl₄ was around 1:1. Therefore, the reason for the failure to observe fluorescent AuNCs using A₃₀ in Figure 1B was due to the too high DNA concentration. This binding stoichiometry suggests the high affinity between adenine and gold. We next fixed A₃₀ at 4 μM and varied HAuCl₄ concentration. The highest fluorescence was observed with 100-150 μM HAuCl₄ (Figure 3C). A further increase of HAuCl₄ produced red/purple colored nanoparticles. Next the effect of pH was tested and the highest fluorescence was observed using an A₃₀:HAuCl₄ ratio of 1:30 at pH 6 (Figure 3B), where adenine was not protonated. The decreased fluorescence at lower pH suggests that gold binding might involve the N7 position of adenine (Figure S3), whose *pK_a* is ~3.5. The optimal citrate concentration was determined to be 50 mM or higher (Figure S7). A photograph was also taken for the pH samples (Figure 3D). Interestingly, we observed a slight red fluorescence at pH 4. The synthesis of AuNCs at various pH in the presence of pepsin was recently reported, where a broad range of emission colors were obtained.¹⁹

The AuNCs prepared with A₃₀ has a slightly longer emission peak centered at ~480 nm (Figure 3E). The excitation spectrum shows a peak at 280 nm, which is 20 nm longer than the absorption peak of poly-A DNA. To understand this, we measured the absorption spectra of the sample and the absorption maximum was at 270 nm (Figure S8), similar to the excitation peak. The small peak 413 nm was mainly due to the water Raman scattering, where water alone can generate the same feature.

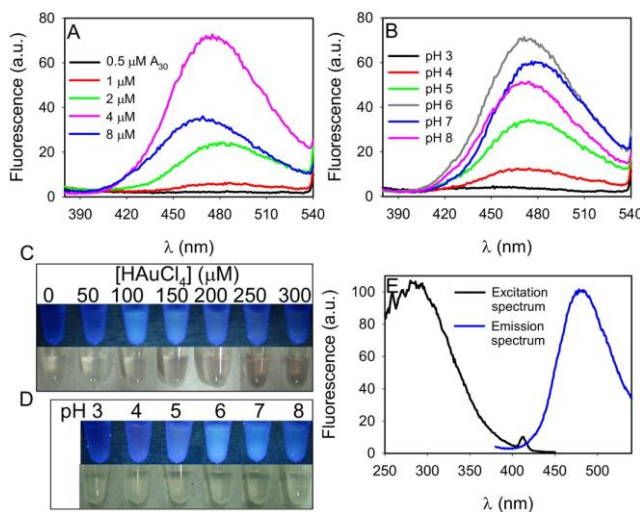


Figure 3. Fluorescence spectra of AuNCs synthesized using various concentrations of A₃₀ at pH 7 (A) and using different pH (B). [HAuCl₄]=150 μM for all the samples. Photographs of AuNCs synthesized with increasing HAuCl₄ concentration (C) and at various pH (D). (E) Fluorescence emission and excitation spectra of AuNCs templated by A₃₀.

While DNA has been used to control the synthesis of AuNPs,^{20,21} this is the first report on making fluorescent AuNCs. This study has revealed the binding interaction between gold and cytosine at low

pH. This interaction is weaker than that between silver and cytosine but still strong enough to allow controlled reduction of HAuCl₄. Poly-A DNA requires a more strict control of the ratio with HAuCl₄ and it works better at around neutral pH, where adenine is not protonated. These AuNCs can receive energy from nucleobases to boost their emission intensity. The resulting AuNCs could be used for various analytical and biomedical applications. Future work will be focused on optimizing DNA sequences and synthesis conditions to further improve quantum yield and to achieve other emission colors.

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Notes and references

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Electronic Supplementary Information (ESI) available: materials and methods, TEM micrograph, additional UV-vis and fluorescence spectra, kinetics, and gold/DNA interactions. See DOI...

1. J. Zheng, P. R. Nicovich and R. M. Dickson, *Annu. Rev. Phys. Chem.*, 2007, **58**, 409; (b) R. C. Jin, *Nanoscale*, 2010, **2**, 343; (c) J. F. Parker, C. A. Fields-Zinna and R. W. Murray, *Acc. Chem. Res.*, 2010, **43**, 1289; (d) L. Shang, S. J. Dong and G. U. Nienhaus, *Nano Today*, 2011, **6**, 401.
2. Z. Wu and R. Jin, *Nano Lett.*, 2010, **10**, 2568.
3. J. Zheng, C. Zhang and R. M. Dickson, *Phys. Rev. Lett.*, 2004, **93**, 077402.
4. R. C. Hedden, B. J. Bauer, A. Paul Smith, F. Grohn and E. Amis, *Polymer*, 2002, **43**, 5473.
5. Y. Bao, C. Zhong, D. M. Vu, J. P. Temirov, R. B. Dyer and J. S. Martinez, *J. Phys. Chem. C*, 2007, **111**, 12194.
6. T. G. Schaaff and R. L. Whetten, *J. Phys. Chem. B*, 2000, **104**, 2630.
7. J. P. Xie, Y. G. Zheng and J. Y. Ying, *J. Am. Chem. Soc.*, 2009, **131**, 888; (b) L. Yan, Y. Q. Cai, B. Z. Zheng, H. Y. Yuan, Y. Guo, D. Xiao and M. M. F. Choi, *J. Mater. Chem.*, 2012, **22**, 1000; (c) Z. Q. Yuan, M. H. Peng, Y. He and E. S. Yeung, *Chem. Comm.*, 2011, **47**, 11981; (d) C. L. Liu, H. T. Wu, Y. H. Hsiao, C. W. Lai, C. W. Shih, Y. K. Peng, K. C. Tang, H. W. Chang, Y. C. Chien, J. K. Hsiao, J. T. Cheng and P. T. Chou, *Angew. Chem. Int. Ed.*, 2011, **50**, 7056; (e) X. Le Guevel, N. Daum and M. Schneider, *Nanotechnology*, 2011, **22**, 275103; (f) P. L. Xavier, K. Chaudhari, P. K. Verma, S. K. Pal and T. Pradeep, *Nanoscale*, 2010, **2**, 2769; (g) F. Wen, Y. H. Dong, L. Feng, S. Wang, S. C. Zhang and X. R. Zhang, *Anal. Chem.*, 2011, **83**, 1193; (h) X. Le Guevel, B. Hotzer, G. Jung, K. Hollemeyer, V. Trouillet and M. Schneider, *J. Phys. Chem. C*, 2011, **115**, 10955.
8. Ono, S. Cao, H. Togashi, M. Tashiro, T. Fujimoto, T. Machinami, S. Oda, Y. Miyake, I. Okamoto and Y. Tanaka, *Chem. 75 Comm.*, 2008, 4825.
9. J. T. Petty, J. Zheng, N. V. Hud and R. M. Dickson, *J. Am. Chem. Soc.*, 2004, **126**, 5207; (b) C. I. Richards, S. Choi, J.-C. Hsiang, Y. Antoku, T. Vosch, A. Bongiorno, Y.-L. Tzeng and R. M. Dickson, *J. Am. Chem. Soc.*, 2008, **130**, 5038; (c) E. G. Gwinn, P. O'Neill, A. J. Guerrero, D. Bouwmeester and D. K. Fygenson, *Adv. Mater.*, 2008, **20**, 279; (d) G. Y. Lan, W. Y. Chen and H. T. Chang, *Rsc Advances*, 2011, **1**, 802; (e) B. Y. Han and E. K. Wang, *Anal.*

- Bioanal. Chem.*, 2012, **402**, 129; (f) W. Y. Chen, G. Y. Lan and H. T. Chang, *Anal. Chem.*, 2011, **83**, 9450.
10. J. Liu, Z. Cao and Y. Lu, *Chem. Rev.*, 2009, **109**, 1948.
 11. W. W. Guo, J. P. Yuan, Q. Z. Dong and E. K. Wang, *J. Am. Chem. Soc.*, 2010, **132**, 932; (b) L. Deng, Z. X. Zhou, J. Li, T. Li and S. J. Dong, *Chem. Comm.*, 2011, **47**, 11065; (c) T. Li, L. B. Zhang, J. Ai, S. J. Dong and E. K. Wang, *ACS Nano*, 2011, **5**, 6334. (d) W. W. Guo, J. P. Yuan and E. K. Wang, *Chem. Comm.*, 2011, **47**, 10930; (e) H. C. Yeh, J. Sharma, J. J. Han, J. S. Martinez and J. H. Werner, *Nano Lett.*, 2010, **10**, 3106; (f) J. J. Yin, X. X. He, K. M. Wang, Z. H. Qing, X. Wu, H. Shi and X. H. Yang, *Nanoscale*, 2012, **4**, 110.
 12. M. Ritchie, K. R. Johnsen, J. R. Kiser, Y. Antoku, R. M. Dickson and J. T. Petty, *J. Phys. Chem. C*, 2007, **111**, 175.
 13. X. Zhang, M. R. Servos and J. Liu, *Langmuir*, 2012, **28**, 3896; (b) X. Zhang, M. R. Servos and J. Liu, *J. Am. Chem. Soc.*, 2012, **134**, 7266.
 14. P. R. O'Neill, E. G. Gwinn and D. K. Fygenson, *J. Phys. Chem. C*, 2011, **115**, 24061.
 15. J. J. Storhoff, R. Elghanian, C. A. Mirkin and R. L. Letsinger, *Langmuir*, 2002, **18**, 6666.
 16. H. Kimura-Suda, D. Y. Petrovykh, M. J. Tarlov and L. J. Whitman, *J. Am. Chem. Soc.*, 2003, **125**, 9014.
 17. M. S. Holowczak, M. D. Stancil and G. B. Wong, *J. Am. Chem. Soc.*, 1985, **107**, 5789.
 18. S. Shirotake and T. Sakaguchi, *Chem. Pharm. Bull.*, 1977, **25**, 3232.
 19. H. Kawasaki, K. Hamaguchi, I. Osaka and R. Arakawa, *Adv. Funct. Mater.*, 2011, **21**, 3508.
 20. Z. Wang, J. Zhang, J. M. Ekman, P. J. A. Kenis and Y. Lu, *Nano Lett.*, 2010, **10**, 1886.
 21. L. Xu, Y. Zhu, W. Ma, W. Chen, L. Liu, H. Kuang, L. Wang and C. Xu, *J. Phys. Chem. C*, 2011, **115**, 3243.